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(71) Applicant(s)
Asahi Kogaku Kogyo Kabushiki Kaisha

(Incorporated in Japan)

No 36-9 Maenocho 2-chome, Itabashi-ku, Tokyo, Japan

Tadahiko Kitano
954-30 Yokokawamachi Hachioji-shi, Tokyo, Japan

Mikio Nakayama
11-25 Saginumadai 3-chome, Narashino-shi, Chiba-ken, Japan

(72) Invantor(s)
Tadahiko Kitano
Mikio Nakayama
Ayumi Mito

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(72) cont
Tetsuro Ogawa
Tsuneo Hiraide

(74) Agent and/or Address for Sarvica
Ablett & Stebbing
45 Lancaster Mews, Lancaster Gate, LONDON, W2 3QQ, United Kingdom

(54) **Detection sheet comprising calcium phosphate particles**

(57) There is provided a detection sheet comprising a known antigen or antibody immobilized on a fibrous composite having carried thereon particles of a calcium phosphate compound e.g. tricalcium phosphate, hydroxyapatite or flourapatite, having an average particle diameter of 0.01 to 200 microns and a Ca/P ratio of 1.0 to 2.0. Using this detection sheet, the detection is made by contacting the sheet with a test medium anticipated to show the presence of an antigen or antibody, thereby bonding the anticipated antigen or antibody to said known antigen or antibody of said sheet, and then detecting the resulting antigen-antibody complex upon contact of said complex with a solution of a labeling compound capable of specifically binding to said anticipated antigen or antibody.

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SHEETS, KITS AND METHODS FOR DETECTING ANTIGENS OR ANTIBODIES

The present invention relates to a detection sheet, detection kit and detection method for detecting an antigen or antibody in a test medium such as biological fluid, for example, saliva, blood, lymph and other fluids.

Recently, in the medical field, it has become important to conduct clinical examinations by means of an external or extracorporeal diagnostic process. In this external diagnostic process well-known methods for detecting antigens or antibodies include , for example, radioimmunoassay, enzyme immunoassay and others. However, since it uses a radioisotope, radioimmunoassay can not be generally utilized in hospitals and other facilities. Further, in clinics or smaller hospitals, it is difficult to conduct such an immunoassay immediately upon request, because they sometimes employ no medical technologist or expert. As an alternative, it is possible for such clinics or hospitals to conduct such an immunoassay by entrusting the examination to other organizations. However, this requires an extended period of

time until they receive the results of the examination. It is, therefore, desirable to develop a novel material or article for use in the diagnosis of various diseases, which enables the provision of a result of the examination rapidly and with a high sensitivity by a simplified process.

The object of the present invention is to provide a detection sheet, detection kit and detection method, according to which an antigen or antibody contained in a test medium such as biological fluid can be rapidly detected with a high detection sensitivity in a simplified operation. The other objects of the present invention will be appreciated from the description as set forth below with regard to the preferred embodiments thereof.

According to one aspect of the present invention, the above object can be attained by a detection sheet for detecting an antigen or antibody anticipated to be contained in a test medium, which sheet comprises a known antigen or antibody immobilized on a fibrous composite comprising particles of a calcium phosphate compound having an average particle diameter of 0.01 to 200 microns and a molar ratio of calcium to phosphorus (hereinafter, Ca/P ratio) of 1.0 to 2.0.

According to another aspect of the present invention, the above object can be attained by a detection kit for detecting an antigen or antibody anticipated to be contained in a test medium, which kit comprises the detection sheet according to the present invention and a solution of a labeling compound for the anticipated antigen or antibody to be associated with said detection sheet.

According to another aspect of the present invention, the above object can be attained by a method for detecting an antigen or antibody anticipated to be contained in a test medium, which method comprises the steps of:

immobilizing a known antigen or antibody on a fibrous composite comprising particles of a calcium phosphate compound having an average particle diameter of 0.01 to 200 microns and a Ca/P ratio of 1.0 to 2.0 to form a detection sheet,

masking the antigen- or antibody-unadsorbed sites of the detection sheet with a blocking agent having an adsorptivity to the calcium phosphate compound and comprising at least one protein having a low specificity to the immobilized antigen or antibody,

contacting said detection sheet with said test medium to induce a reaction between said immobilized antigen or antibody and said anticipated antigen or antibody, and

further contacting said detection sheet containing therein an antigen-antibody complex formed in said reaction with a solution of a labeling compound capable of specifically binding to said anticipated antigen or antibody to detect said antigen-antibody complex.

As will be further appreciated from the following description of the preferred embodiments thereof, the present invention is based on the finding that satisfactory detection of antigens and antibodies can be attained if an antigen-antibody reaction in an immunoassay is made on a sheet-like article comprising a fibrous composite having carried thereon particles of the specified calcium phosphate compound having an adsorptivity to the antigens and antibodies. The particles of the calcium phosphate compound used herein, as described above and will be further described hereinafter, have an average particle diameter of 0.01 to 200 microns and a Ca/P ratio of 1.0 to 2.0.

Using the sheet-like article or detection sheet of the present invention, it becomes possible to detect any anticipated antigen or antibody from a test medium by the simplified steps of operation, with rapidity and, with a high detection sensitivity. In addition, there is provided a low-cost detection kit which allows a easy, rapid and exact detection of the antigen or

antibody at clinics or smaller hospitals not employing experts such as medical technicians and the like. Note that the term "test medium" used herein is intended to mean a wide variety of fluid materials to which the present invention can be advantageously applied. Typical examples thereof include a biological fluid, for example, saliva, blood, lymph and other liquids.

In the detection sheet according to the present invention, the particles of the calcium phosphate compound carried on the fibrous composite are used as an agent for adsorbing and immobilizing a known antigen or antibody on the composite. The calcium phosphate compounds used herein are not limited insofar as they show a Ca/P ratio in the range of 1.0 to 2.0. Accordingly, the calcium phosphate compounds can be optionally selected from a wide variety of useful well-known calcium phosphate compounds depending upon particulars of the detection sheet and other factors. For example, one or more of $Ca_{10}(PO_4)_6(OH)_2$, $Ca_{10}(PO_4)_6F_2$, $Ca_{10}(PO_4)_6Cl_2$, $Ca_3(PO_4)_2$, $Ca_2P_2O_7$, $Ca_4O(PO_4)_2$ and $CaHPO_4$ may be used as the calcium phosphate compounds. Among these calcium phosphate compounds, hydroxyapatite and tricalcium phosphate are preferably used, and the most preferred one is a calcium phosphate compound which contains

hydroxyapatite as a principal component thereof. When fluoroapatite is used as a calcium phosphate compound, it is preferred that the percentage of fluorine in all the calcium phosphate compounds is not more than 5% by weight, because the fluorine content above 5% by weight can result an undesirable elution of fluorine from such compounds. The calcium phosphate compounds may be produced in any conventional manner including a wet process, a dry process and other processes.

The particles of the calcium phosphate compound can be produced in any conventional manner . For example, they can be produced by spray-drying a slurry of the calcium phosphate compound and then sintering the dried product. It is also possible to use other granulation methods in the production of the particles of the calcium phosphate compound, if desired. Preferably, sieve and other separation means may be used to selectively obtain the particles of the calcium phosphate compound having the predetermined range of the particle size depending on the intended use of the particles.

It is preferred that the particles of the calcium phosphate compound have an average particle diameter of 0.01 to 200 microns. The average particle diameter of less than 0.01 microns will cause too easy an aggregation of the particles, thereby inhibiting formation of the uniformly

carried particles on the fibrous composite such as nonwoven fabric, while an average particle diameter of more than 200 microns will produce insufficient particles on the fibrous composite, i.e., it will show a notably reduced percentage of the particles on the composite.

Further, the particles of the calcium phosphate compound are preferably porous particles. More particularly, the porous particles may comprise agglomerated primary particles having a specific surface area of not less than 10 m^2/g and pore size of 500 to 1000 angstroms. A specific surface area of less than 10 m^2/g should be avoided, because such a specific surface area does not ensure a satisfactory adsorptivity. Also, in order to attain. introduction of the adsorbed proteins and other substances into pores or cells of the particles, it is preferred that the porous particles contain pores or cells having the above-defined pore size of 500 to 1000 angstroms.

The porous particles of the calcium phosphate compound can be produced according to any conventional method . For example, they can be produced from starting particles which are crystalline particles of calcium phosphate compounds synthesized in a well-known wet process. A slurry of the starting particles as a suspension is directly spray-dried or subjected to other treatments to form

secondary particles or is spray-dried or treated according to other methods to form secondary particles after addition of additives, such as a viscosity modifier and particles or fibers of an organic compound capable of being dissipated upon heating, to the slurry.

The resulting secondary particles already have a porous structure and accordingly they may be used as a starting material in the production of the detection sheet. Alternatively, if it is desired to obtain porous particles of the calcium phosphate compounds having a highly increased porosity (porous granules), such porous granules can be produced by preparing a slurry of the secondary particles as a suspension and then molding the slurry in a wet process or in a dry process including application of the pressure, to produce a block body of the calcium phosphate compounds. In the preparation of the slurry, any organic compound which may be dissipated from the block body during the subsequent sintering process may be added to the slurry in order to assist formation of finely divided pores or cells in the resulting granules. Of course, addition of such organic compound is optional, and it may be omitted if not desired, because a pore size or diameter of the granules can be controlled by changing the applied sintering temperature and other conditions. The obtained block body is then sintered at a temperature of

500 to 1300 ℃. A temperature of less than 500℃ is insufficient to complete thermal dissipation of the organic compound and sintering of the block body. And, if sintering of the block body is carried out at an elevated temperature of more than 1300 ℃, an excessively dense sintered body can be produced or decomposition of the calcium phosphate can be caused. The thus sintered block body is pulverized and then classified to obtain porous granules having a desired particle size. The pore size of the porous granules can be controlled by suitably varying the size of the crystalline particles of the calcium phosphate compounds in the starting slurry for use in the preparation of the secondary particles. The viscosity of the slurry, the particulars of additives and other factors.

In the detection sheet of the present invention, the above-described particles of the calcium phosphate compound are carried on a fibrous composite. The fibrous composite used herein includes a wide variety of composites of the fibrous materials, and typical examples of useful fibrous composites include paper and nonwoven fabrics of naturally occurring materials or synthetic materials.

The formation of the fibrous composite comprising the particles of the calcium phosphate compound can be carried out by using any conventional paper making or sheet making methods, for example. If the fibrous composite is a

paper, the paper carried with the particles of the calcium phosphate compound can be produced, for example, by using particles of the calcium phosphate compound as a filler and adding this filler to the paper making material in an internal addition or incorporation method, or adding the filler to the raw paper in a coating method. If the filler-incorporation method is used in the production of the carrier paper, the particles of the calcium phosphate compound and other additives can be added to the paper making material which, after thorough mixing, is passed through a conventional paper making machine to produce the carrier paper. Further, if the filler-coating method is used, the particles of the calcium phosphate compound along with a binding agent can be coated over the raw paper to produce the carrier paper. The binding agent used herein is not restrictive, and typical examples thereof include sodium polyacrylate, polyvinylalcohol, latex, polyacrylic acid, polyethyleneoxide, carboxymethylcellulose, polyester and the like.

As in the above-described production of the carrier paper, similar methods can be used in the production of the nonwoven fabric carried with the particles of the calcium phosphate compound. More preferably, the nonwoven carrier fabric can be produced by applying particles of the calcium phosphate compound on at least one surface of the nonwoven

fabric made of the fibers, at least a part of which fibers consists of thermoplastic polymeric fibers, in a wet process or a dry process. After formation of the nonwoven carrier fabric, a thermal treatment is used to soften at least a surface portion of the polymeric fibers in the nonwoven fabric, thereby fixing the particles of the calcium phosphate compound to said surface portion of the fibers.

In the fibrous composite comprising particles of the calcium phosphate compound, the amount of the carried particles is generally in the range of 1 to 65 % by weight, preferably in the range of 5 to 50 % by weight. If the amount of the carried particles of the calcium phosphate compound is less than 1 % by weight, an adsorption of an antigen or antibody on the composite can be attained only with difficulty. However, if it exceeds 65 % by weight, expense can be increased, because the amount of the antigen and antibody used, as well as the concentration of the blocking agent used, are increased.

In the thus produced fibrous composite with the carried particles, at least a surface portion of the composite, as described above, contains a layer of the calcium phosphate compound and accordingly the fibrous composite can exhibit an exellent adsorptivity to antibodies and antigens such as bacteria, viruses and the like. Namely, the fibrous composite with the carried

particles used in the detection sheet of the present invention can effectively adsorb the antigens or antibodies.

The detection sheet of the present invention can be produced by adsorbing a known antigen or antibody onto the fibrous composite with the carried particles and then immobilizing the adsorbed antigen or antibody to form an immobilized antigen or antibody. Note that the antigens and antibodies used herein for the immobilization purpose are not restrictive, and can be freely selected from a wide variety of the well-konwn antigens and antibodies depending on the particulars of the anticipated antigens and antibodies in the test medium and other factors. Also, the immobilization process used herein is not restrictive, and any conventional immobilization methods can be used.

Any conventional immobilizing agents can be used in the immobilization process. Preferably, a crosslinking agent containing at least one aldehyde or epoxy group in a molecule thereof can be used. Typical examples of suitable immobilizing agents include glutaraldehyde, formaldehyde, 3-glycidoxypropyltrimethoxysilane, bisoxysilane, epichlorohydrin and the like.

After immobilization, it is preferred that the free sites of the detection sheet, to which sites the antigen or antibody has not been adsorbed, is treated with a blocking agent in order to mask said antigen- or antibody-unadsorbed

sites of the detection sheet. The blocking agent used herein is not restrictive, insofar as it can be adsorbed on such antigen- or antibody-unadsorbed sites of the detection sheet and has a less specificity to the immobilized antigen or antibody. However, it is preferred that proteins such as casein, albumin and gelatin are used as a blocking agent.

The thus produced immobilized antigen or immobilized antibody, if it is contacted with a test medium such as test solution in which the presence of any antigen or antibody is anticipated, can easily detect such anticipated antigen or antibody with a high detection sensitivity, after it is further contacted with a solution of suitable labeling compound. Moreover, the above-described masking of the antigen- or antibody-unadsorbed sites of the detection sheet with a suitable blocking agent is effective to diminish the tendency for undesired nonspecific reaction to a remarkably reduced level. Thus, the detection sheet of the present invention containing such immobilized antigen or immobilized antibody can be suitably used in the detection of any antigens or antibodies in a test medium.

In order to improve the handling properties thereof, the detection sheet may further comprise any reinforcing member applied to a back surface of the fibrous composite. The reinforcing member is preferably in the form of film or sheet. The reinforcing member can be preferably produced

from various materials such as paper, plastics, ceramics, metals and others by using any molding and other shaping methods. In addition, according to the present invention, a detection kit for detecting any antigen or antibody can be provided by associating the above-described detection sheet of the present invention and a solution of a labeling compound for the anticipated antigen or antibody. Note that the term "association" used herein is intended to mean that in the practice of the present invention, any combination of the detection sheet and the solution of the labeling compound can be used in order to obtain the desired detection kit.

The labeling compound used in the preparation of the detection kit can be selected from various labeling compounds well-known in the field of immunoassay. Typical examples of useful labeling compounds include enzyme-labeled antibodies or antigens, isotope-labeled antibodies or antigens and others, which are able to be specifically bonded with an anticipated antigen or antibody in a test medium. It is preferred to use the enzyme-labeled antibodies or antigens as the labeling compound, because they do not require any specially designed installations which are essential to use of the isotope-labeled antibodies or antigens, in addition to simplified detection.

Further, when the enzyme-labeled antibodies or antigens are used as the labeling compound, it is contemplated that a substrate for said labeling enzyme capable of indicating a color at a wavelength of visible or ultraviolet radiation is used in combination with said labeling compound so that the anticipated antigen or antibody can be easily detected by using any simplified methods such as visual inspection, determination of absorbance and the like. Suitable combinations of the enzyme and the substrate include , for example:

enzyme	substrate
alkaline phosphatase	BCIP and NBT
ditto	DNP
horseradish peroxidase	OPD
ditto	DAB
ditto	4CN
β -D-galactosidase	pNPG
ditto	X-gal
ditto	Bluo-gal

The detection sheet and detection method according to the present invention can be principally used to ascertain whether or not an antigen or antibody is contained in a test medium. Further, they can be applied to a quantitative analysis of the antigen or antibody with satisfactory results, if a comparison in the color density between the

indicated colors or a determination of the absorbance is made following the detection step.

The present invention will be further described with reference to some working examples thereof. Note, however, that the present invention is not restricted by these examples.

EXAMPLE 1

Preparation of Detection Sheet:

Porous particles of hydroxyapatite having an average particle diameter of 3.5 microns and a Ca/P ratio of 1.67 were applied to a nonwoven fabric having a thickness of 0.2 mm and a size of 5 mm × 10 mm consisting of 50 % by weight of polyethylene and 50 % by weight of polyethylene terephthalate, and the nonwoven fabric was thermally treated to produce a ceramics/fibrous composite having substantially uniformly carried thereon 24 % by weight of the hydroxyapatite granules.

A 256 HA (hemagglutination) value of A-type influenza virus was adsorbed onto the fibrous composite, and the composite immersed in a four times-diluted solution of a blocking agent containing casein, trade name "Block Ace" commercially available from Snow Brands Milk Products Co., Ltd. in order to selectively mask the influenza virus-unadsorbed sites of the composite. The detection sheet of the present invention, i.e., influenza

virus-bonded fibrous composite, was thus produced.

Evaluation of the Detection Sheet:

The obtained detection sheet was evaluated with regard to a virus adsorption sensitivity thereof. The detection sheet was immersed in a varied dilution of rabit antiserum to the above-mentioned influenza virus (test medium), and its virus adsorption sensitivity was determined by examining an enzyme immunoassay using an alkaline phosphatase-bonded antibody and DNP as the substrate. The decision of "positive" or "negative" was made on an absorption meter, Microwell system, commercially available from Organon Teknika Co. The decision "positive" was assigned to the tested sheet, when it indicated an absorbance of 0.2 or more, i.e., it indicated a yellow color. Note that the decision was made twice for each sheet, and the first decision was made 10 minutes after the initiation of said enzyme-antibody reaction and the second decision was made one hour after.

For comparison purposes, the above procedure was repeated by using a varied dilution of normal rabit serum in place of the dilution of the above-described antiserum.

The results of the above evaluation tests evidenced that the detection sheet embodying the present invention has an exellent sensitivity and specificity. Thus, for the evaluation test using the antiserum to the influenza

virus, in the 10 minutes after initiation of the reaction, the decision "positive" was detectable until the antiserum dilution was further diluted by a factor of 6400.

One hour after initiation of the reaction, the decision "positive" was detectable until the antiserum dilution was further diluted by a factor of 51200 times or more. Contrary to these satisfactory results, for the evaluation test using the normal serum, the decision "negative" was assigned at a factor of dilution of only 10 times or less.

EXAMPLE 2

Preparation of Detection Sheet:

Porous particles of hydroxyapatite having an average particle diameter of 80 microns and a Ca/P ratio of 1.67 were applied along with sodium polyacrylate as a binding agent to a nonwoven fabric having a thickness of 0.3 mm and a size of 5 mm× 10 mm consisting of 100 % by weight of rayon to produce a ceramics/fibrous composite having substantially uniformly carried thereon 16 % by weight of hydroxyapatite granules.

A 256 HA value of A-type influenza virus was immobilized on the fibrous composite in the manner similar to that of Example 1 to produce a detection sheet of the present invention, i.e., influenza virus-bonded fibrous composite.

Evaluation of the Detection Sheet:

The procedure of Example 1 was repeated by using the detection sheet (influenza virus-bonded fibrous composite) produced in the above step. The test medium was a 100 × dilution of rabit antiserum to the influenza virus, and the control for a comparative purpose a 100 × dilution of normal rabit serum, respectively. The results of the evaluation tests evidenced that for the evaluation test using the antiserum to the influenza virus, a fully clear positive reaction could be observed, while a strength of the color indication of the substrate was reduced to about one half of that obtained by using the influenza virus-bonded fibrous composite of Example 1. For the evaluation test using the normal serum, it was apparent that the decision "negative" should be assigned.

EXAMPLE 3

Preparation of Detection Sheet:

Porous particles of tetracalcium phosphate having an average particle diameter of 4.0 microns and a Ca/P ratio of 2.0 were applied to a nonwoven fabric having a thickness of 0.2 mm and a size of 5 mm × 10 mm consisting of 100 % by weight of polypropylene, and the nonwoven fabric was thermally treated to produce a ceramics-carried fibrous composite having substantially uniformly carried thereon 28 % by weight of the tetracalcium phosphate granules.

A 256 HA value of A-type influenza virus was immobilized on the fibrous composite in the manner similar to that of Example 1 to produce the detection sheet of the present invention, i.e., influenza virus-bonded fibrous composite.

Evaluation of the Detection Sheet:

The procedure of Example 2 was repeated. The results of the evaluation tests evidenced that the results were slightly less good in comparison with the results obtained by using the influenza virus-bonded fibrous composite of Example 1, however, the detection itself was not impaired.

EXAMPLE 4

Preparation of Detection Sheet:

Porous particles of tricalcium phosphate having a particle diameter of 100 to 200 microns and a Ca/P ratio of 1.5 were applied to a nonwoven fabric having a thickness of 0.4 mm and a size of 5 mm × 10 mm consisting of 50 % by weight of polyethylene and 50 % by weight of polyethylene terephthalate, and the nonwoven fabric was thermally treated to produce a ceramics/fibrous composite having substantially uniformly carried thereon 28 % by weight of the tetracalcium phosphate granules.

A 256 HA value of A-type influenza virus was immobilized on the fibrous composite in the manner similar

to that of Example 1 to produce a detection sheet of the present invention, i.e., influenza virus-bonded fibrous composite.

Evaluation of the Detection Sheet:

The procedure of Example 2 was repeated. The results of the evaluation tests were good, and were comparable to the results obtained by using the influenza virus-bonded fibrous composite of Example 1.

EXAMPLE 5

Preparation of Detection Sheet:

Porous particles of monocalcium phosphate having an average particle diameter of 0.05 microns and a Ca/P ratio of 1.0 were applied along with polyvinyl alcohol as a binding agent to a nonwoven fabric having a thickness of 0.1 mm and a size of 5 mm × 10 mm consisting of 100 % by weight of polyethylene terephthalate to produce a ceramics/fibrous composite having substantially uniformly carried thereon 16 % by weight of the hydroxyapatite granules.

A 256 HA value of A-type influenza virus was immobilized on the fibrous composite in the manner similar to that of Example 1 to produce the detection sheet of the present invention, i.e., influenza virus-bonded fibrous composite.

Evaluation of the Detection Sheet:

The procedure of Example 2 was repeated. The results of the evaluation tests evidenced that the sensitivity was slightly reduced in comparison with the results obtained by using the influenza virus-bonded fibrous composite of Example 1, however, a satisfactory assay was made.

EXAMPLE 6

Preparation of Detection Sheet:

A suspension containing 0.7% by weight of conifer wood pulp and 0.3 % by weight of porous particles of hydroxyapatite having an average particle diameter of 3.5 microns and a Ca/P ratio of 1.67 was subjected to a paper making process in accordance with the instructions defined under JIS-P8209 to produce a ceramics/fibrous composite (size of 5 mm × 10 mm) having substantially uniformly carried thereon 30 % by weight of hydroxyapatite.

A 256 HA value of A-type influenza virus was immobilized on the fibrous composite in the manner similar to that of Example 1 to produce the detection sheet of the present invention, i.e., influenza virus-bonded fibrous composite.

Evaluation of the Detection Sheet:

The procedure of Example 2 was repeated. The results of the evaluation tests were good, and were comparable to the results obtained by using the influenza virus-bonded fibrous composite of Example 1.

EXAMPLE 7

The procedure of Example 1 was repeated except that for this example, 40 HA value of Japanese encephalitis virus was immobilized on the fibrous composite, in place of 256 HA value of A-type influenza virus. The results of the evaluation tests evidenced that for the dilution of the antiserum, in the 10 minutes after initiation of the reaction, the decision "positive" was detectable down to a dilution factor of 3200 times. One hour after initiation of the reaction, the decision "positive" was detectable down to a dilution factor of 51200 times or more. Further, in the dilution of the normal serum, in both the 10 minute assay and the one hour after initiation assay of the enzyme-antibody reaction, no nonspecific positive reaction was observed, even if a low dilution having a dilution factor of only 20 times or less was used.

EXAMPLE 8

The procedure of Example 1 was repeated except that for this example, 256 HA value of rabies virus was immobilized on the fibrous composite, in place of 256 HA value of A-type influenza virus. The results of the evaluation tests evidenced that for the dilution of the antiserum, in the 10 minutes after initiation of the enzyme-antibody reaction, the decision "positive" was detectable down to a dilution factor of 200 times. One hour

after initiation of the reaction, the decision "positive" was detectable down to a dilution factor of 51200 times or more. Further, for the dilution of the normal serum, in both the 10 minute assay and the one hour after initiation assay of the reaction, no nonspecific positive reaction was observed, even at a dilution factor of only 20 times or less.

COMPARATIVE EXAMPLE 1

The test plate for the ELISA, Linbro/Titertek, commercially available from Linbro Co., and a detection sheet consisting of a hydroxyapatite-carried fibrous composite having a size of 5 mm x 10 mm used in Example 1 were used in the comparison tests in order to ascertain any difference in the sensitivity between the plate and the sheet.

The A-type influenza virus, Japanese encephalitis virus and rabies virus were separately adsorbed on each the test plates and the detection sheet in accordance with the method of Example 1, and the titer of the antiserum was determined. The results evidenced that the detection sheet of Example 1 exhibited a sensitivity which is equivalent to or higher than that of the test plate for the ELISA.

COMPARATIVE EXAMPLE 2

Two types of nonwoven fabrics, i.e., a non-carrier nonwoven fabric having a thickness of 0.2 mm and a size of

20 mm × 20 mm consisting of 50 % by weight of polyethylene and 50 % by weight of polyethylene terephthalate, and a carrier nonwoven fabric of the same thickness and size as well as material, i.e., the hydroxyapatite-carried nonwoven fabric comprising 22 % by weight of the hydroxyapatite granules substantially uniformly carried thereon and produced in the manner described in Example 1, were used in comparison tests in order to ascertain effectiveness in virus adsorption of the hydroxyapatite-carrier nonwoven fabric.

The adsorption of the virus on the non-carrier and carrier nonwoven fabrics was made in the manner similar to that of Example 1. The test virus used herein was a floating solution of A-type influenza virus, and, as will be appreciated from Table 1, the virus dilutions used had four different dilution factors, i.e., 10, 20, 40 and 80 times, and accordingly four different HI titers, i.e., 1024, 512, 256 and 128, respectively. Note that HI is an abbreviation of hemagglutination inhibition.

Each nonwoven fabric was immersed in 1 ml of the above-mentioned virus dilution, and shaken at a room temperature for one hour. A supernatant was separated, and was subjected to a hemagglutination test using erythrocyte (red blood cells) of chickens. The results of the test are summarized in the following Table 1. Note, in Table 1,

that the control contained only a floating solution of A-type influenza virus.

TABLE 1

dilution degree	titer of supernatant after contact with apatite-carrier nonwoven	with non-carrier nonwoven fabric	control
10	128	512	1024
20	16	256	512
40	4	64	256
80	<4	16	128

It will be appreciated from the results of Table 1 that the hydroxyapatite-carrier nonwoven fabric has a notably excellent virus adsorption power.

COMPARATIVE EXAMPLE 3

Two types of nonwoven fabrics, i.e., the non-carrier nonwoven fabric having a thickness of 0.2 mm and a size of 20 mm × 20 mm consisting of 50 % by weight of polyethylene and 50 % by weight of polyethylene terephthalate, and the carrier nonwoven fabric of the same thickness and size as well as material, i.e., the hydroxyapatite-carried nonwoven fabric comprising 22 % by weight of the hydroxyapatite granules substantially uniformly carried

thereon, produced in the manner described in Example 1, were used in the comparison tests in order to ascertain effectiveness in virus adsorption of the hydroxyapatite-carried nonwoven fabric.

The assay, i.e., the test for adsorption of the virus on the non-carrier and carrier nonwoven fabrics, was made in the manner similar to that of Example 1. The test virus used herein was a floating solution of A-type influenza virus, five-times dilution having a HI titer of 2048. The test medium was a 1000 times dilution of a human serum which exhibits a positive reaction in the determination on an ELIZA test plate until the dilution factor falls below 1600 times. The results showed that the hydroxyapatite-carried nonwoven fabric indicated a positive reaction, while the non-carried nonwoven fabric indicated an apparent negative reaction.

CLAIMS

1. A detection sheet for detecting an antigen or antibody anticipated to be contained in a test medium, which sheet comprises a known antigen or antibody immobilized on a fibrous composite comprising particles of a calcium phosphate compound having an average particle diameter of 0.01 to 200 microns and a molar Ca/P ratio of 1.0 to 2.0.

2. A detection sheet according to claim 1 wherein said antigen or antibody is immobilized with a crosslinking agent having at least one aldehyde or epoxy group.

3. A detection sheet according to claim 1 or claim 2 wherein the antigen- or antibody-unadsorbed sites have been masked with a blocking agent having an adsorptivity to the calcium phosphate compound and containing at least one protein having a low specificity to the immobilized antigen or antibody.

4. A detection sheet according to claim 3 which further comprises a reinforcing member applied onto a surface of said fibrous composite.

5. A detection sheet according to claim 4 in which said reinforcing member is in the form of film or sheet.

6. A detection kit for detecting an antigen or

antibody anticipated to be contained in a test medium, which kit comprises a detection sheet and a solution of a labeling compound for the anticipated antigen or antibody for association with said detection sheet, wherein said detection sheet comprises a known antigen or antibody immobilized on a fibrous composite comprising particles of a calcium phosphate compound having an average particle diameter of 0.01 to 200 microns and a Ca/P ratio of 1.0 to 2.0.

7. A detection kit according to claim 6 wherein said antigen or antibody is immobilized with a crosslinking agent having at least one aldehyde or epoxy group.

8. A detection kit according to claim 6 or 7 wherein the antigen- or antibody-unadsorbed sites of the detection sheet have been masked with a blocking agent having an adsorptivity to the calcium phosphate compound and containing at least one protein having a low specificity to the immobilized antigen or antibody.

9. A detection kit according to any of claims 6 to 8 wherein said detection sheet further comprises a reinforcing member having applied a surface of said fibrous composite.

10. A detection kit according to claim 9 wherein said reinforcing member is in the form of film or sheet.

11. A method for detecting an antigen or antibody

anticipated to be contained in a test medium, which method comprises the steps of:

immobilizing a known antigen or antibody on a fibrous composite comprising particles of a calcium phosphate compound having an average particle diameter of 0.01 to 200 microns and a Ca/P ratio of 1.0 to 2.0 to form a detection sheet,

masking the antigen- or antibody-unadsorbed sites of the detection sheet with a blocking agent having an adsorptivity to the calcium phosphate compound and containing at least one protein having a low specificity to the immobilized antigen or antibody,

contacting said detection sheet with said test medium to induce a reaction between said immobilized antigen or antibody and said anticipated antigen or antibody, and

further contacting said detection sheet containing therein an antigen-antibody complex formed in said reaction with a solution of a labeling compound capable of specifically binding to said anticipated antigen or antibody to detect said antigen-antibody complex.

12. A method for detecting an antigen or antibody according to claim 11 in which said antigen or antibody is immobilized with a crosslinking agent having at least one aldehyde or epoxy group.

13. A method for detecting an antigen or antibody

according to claim 11 or 12 wherein said fibrous composite further comprises a reinforcing member having applied to a surface thereof.

The Patent Office

32.

Application No: GB 9518440.4
Claims searched: 1-13

Examiner: Dr. N.R. Curtis
Date of search: 25 October 1995

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Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.N): C3H (HH1); G1B (BAG)

Int Cl (Ed.6): G01N 33/543, 33/551, 33/553

Other: ONLINE: WPI; BIOTECH/DIALOG

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
Y	EP 0,420,053 A1 (W.R. Grace) (See page 2, lines 23-24; page 4, lines 36-52)	1, 3-6, 8-11, 13
Y	WPI Abstract Accession No. 89-088903/12 & JP 010038658 A (Meidensha Elec MFG Co. Ltd.) (See abstract)	1, 3-6, 8-11, 13
Y	WPI Abstract Accession No. 89-088902/12 & JP 010038657 A (Meidensha Elec MFG Co. Ltd.) (See abstract)	1, 3-6, 8-11, 13

X Document indicating lack of novelty or inventive step
Y Document indicating lack of inventive step if combined with one or more other documents of same category.
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E Patent document published on or after, but with priority date earlier than, the filing date of this application.